(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 August 2001 (09.08.2001)

PCT

(10) International Publication Number
WO 01/57183 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/03147

(22) International Filing Date: 31 January 2001 (31.01.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/180,319 4 February 2000 (04.02.2000) US

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(81) Designated States *(national)*: JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHOD FOR PRODUCING CIRCULAR OR MULTIMERIC PROTEIN SPECIES *IN VIVO* OR *IN VITRO* AND RELATED METHODS

(57) Abstract: A method is disclosed for the *in vitro* or *in vivo* cyclization of protein or peptide sequences. Also disclosed is a method of fusing polypeptide sequences while bound to a solid support. These protein manipulation techniques relied on the *trans*-splicing activity of a split intein, such as the naturally occuring split intein form the *dnaE* gene of *Synechocystis* sp. PCC6803 (*Ssp* DnaE intein). The cyclization procedures required the fusion of C- and N-terminal intein splicing domains to the N- and C-termini, respectively, of a target protein ($Intein_C$-target protein-$Intein_N$). Cyclization *in vivo* occurred post-translationally when the two complementary intein splicing domains ligated the N- and C-terminus of the target protein. In vitro cyclization also utilized and $Intein_C$-target protein-$Intein_N$ precursor protein, in which the intein domains were fused to a chitin binding domain (CBD). Protein expression was conducted under conditions that favored the accumulation of precursor protein, which was immobilized on a chitin resin. The circular protein species were eluted from the chitin resin following incubation under conditions that favored protein splicing. *Trans*-splicing was used to ligate polypeptides on a solid support by generating a protein composed of a CBD fused to a C-terminal intein splicing domain and target protein (1). This was incubated with a protein composed of target protein (2) fused to an N-terminal intein splicing domain and a CBD. The precursor proteins were immobilized on a chitin resin where *trans*-splicing resulted in the ligation of target protein (1) to target protein (2). These techniques greatly expand the procedures available for protein engineering and modification.